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Acute and clinically relevant drug-induced liver injury: a population based case-control study

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Aims
To provide quantitative information about the absolute and relative risks of acute and clinically relevant drug-induced liver injury.

Methods
We performed a population-based case-control study using the UK-based General Practice Research Database as the source of information. A total of 1636792 persons subjects aged 5–75 years old registered in the database from 1 January, 1994 to 31 December, 1999 were followed-up for a total of 5404705 person-years. Cases were identified by an exhaustive computer search, then reviewed manually and finally validated against the clinical records. Only idiopathic cases serious enough to be referred to hospital or a consultant were selected. A total of 5000 controls were randomly sampled from the person-time of study cohort. Current users were defined if a prescription ended within 15 days of the index date, and nonusers if there was no prescription before the index date.

Results
One hundred and twenty-eight patients were considered as valid cases, being the crude incidence rate of 2.4 (95% confidence interval: 2.0, 2.8) per 100 000 person-years. The strongest associations were found with chlorpromazine (adjusted odds ratio (AOR); 95% CI = 416; 45, 3840), amoxicillin/clavulanic acid (AOR = 94.8; 27.8, 323), flucloxacillin (AOR = 17.7; 4.4, 71.0), macrolides (AOR = 6.9; 2.3, 21.0), tetracyclines (AOR = 6.2; 2.4, 15.8); metoclopramide (AOR = 6.2; 1.8, 21.3); chlorpheniramine (AOR = 9.6; 1.9, 49.7); betahistine (AOR = 15.3; 2.9, 80.7); sulphasalazine (AOR = 25.5; 6.0, 109); azathioprine (AOR = 10.5; 1.4, 76.4), diclofenac (AOR = 4.1; 1.9, 8.8) and antiepileptics (AOR = 5.1; 1.9, 13.7). A dose-effect was apparent for diclofenac, amoxicillin/clavulanic acid and flucloxacillin. The combination of two or more hepatotoxic drugs increased the risk by a factor of 6. The highest crude incidence rates were found for chlorpromazine, azathioprine, and sulfasalazine (about 1 per 1000 users).

Conclusions
Idiopathic, acute and clinically relevant liver injury, which has the use of drugs as the most probable aetiology, is a rare event in the general population. The relative risks of 40 drugs/therapeutic classes are provided, along with the crude incidence rates for 15 of them where a statistical association was found.

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Introduction

Acute liver injury is nowadays one of the most important pharmacovigilance concerns and the leading cause for drug withdrawal on safety grounds [1]. For most reportedly hepatotoxic drugs, however, the existing information has arisen from individual case reports and an appropriate risk quantification is lacking [2–4]. With the primary aim at providing quantitative information about the absolute and relative risks of acute and clinically relevant drug-induced liver injury, we performed a population-based case-control study using the General Practice Research Database (GPRD) in the UK.

Methods

The primary source of information for this study was the General Practice Research Database that has been described elsewhere [5]. The study population encompassed all subjects aged 5–75 years old with a permanent status registration in the database from 1 January, 1994 to 31 December, 1999. Subjects presenting with a liver-related diagnosis before enrolment were excluded as well as those with cancer, gallbladder or pancreatic disease and alcohol-related disorders. Women who were pregnant during the study period were also excluded. The remaining patients were followed from the start date (1 January, 1994) until the earliest occurrence of one of the following endpoints: a liver-related code diagnosis, age 76 years, death or end of the study period.

We identified all patients with a liver injury code through a broad computer search, and then reviewed all of them by an individual examination of the complete history recorded on computer files. Information on drug exposure was removed to allow for a blinded review by the investigators. A liver injury was defined as an increase of more than two times the upper limit of the normal range in alanine aminotransferase (ALT) or a combined increase in aspartate aminotransferase (AST), alkaline phosphatase (AP) and total bilirubin, provided that one of them was twice the upper limit of the respective normal range [6]. Patients were classified as *noncases* when the liver injury was not confirmed, the patient presented with only minor elevations in serum enzymes, a primary cause was identified (chronic liver disease, malignant neoplasm, viral hepatitis, cholelithiasis, alcoholism, congestive heart failure or other well-defined pathology affecting the liver) or the patient was not referred to a consultant or a hospital (this latter criterion was used to select only clinically relevant cases; by default all patients presenting with jaundice were considered as referred). Patients who died were also excluded because the clinical records were not anymore available at the practice. The remaining patients were classified as *potential cases* and medical records for all of them were requested from the GPs.

After examining the medical records, patients were excluded if they had no confirmed liver injury, antecedents of liver disease, or presented primary causes. The liver injury was classified according to functional criteria [6] as: (a) *hepatocellul*ar; when there was an increase more than twice the upper limit of the normal range in ALT alone or $R \geq 5$, where R is the ratio of serum activity of ALT over serum activity of AP; (b) *cholestatic*; when there was an increase of over twice the upper limit of the normal range in AP alone or $R \leq 2$; or (c) *mixed*; when $2 < R < 5$. The liver injury was considered acute if the clinical and/or laboratory signs had completely disappeared within 6 months from the date of onset.

A total of 5000 controls frequency matched with cases on age (same year), sex and calendar year were sampled from the person-time of the study population. The same exclusion criteria for cases were applied to controls.

Patients were defined as *current users* if a prescription for the drugs of interest or therapeutic class (e.g. antiepileptics, NSAIDs, etc.) lasted until index date or ended within 15 days of the index date (date of onset of liver injury in cases and random date in controls), *past users* if the prescription ended before this period and *nonusers*, if there was no prescription before the index date. For some drugs, a subgroup of *recent users* was defined when the prescription ended between 16 and 30 days before the index day.

We computed the odds ratio (OR) and 95% confidence intervals (95% CI) of acute liver injury associated with current use of individual drugs as compared with nonuse with unconditional logistic regression. We built a first model including the matching factors (age, sex, calendar year), and other variables considered potential confounders such as alcohol consumption (0-1-9, 10 + units/per week, unknown; a unit is equivalent to a glass of wine), smoking (smoker, nonsmoker, exsmoker, unknown) and body mass index [7] (< 25, 25–29.9, 30 + kg m^{-2}, unknown). All drugs showing an OR greater than 2 were included in a second model grouped in four variables (antibiotics, NSAIDs/analgesics, psychotropic/neurological drugs, and a miscellaneous group) in order to adjust for 'other hepatotoxic drugs'.

When the number of cases permitted, the effect of dose and duration of treatment was explored for each drug or class. A stratified analysis was performed by sex

and age (<60, 60+ years) to explore if they were acting as effect modification factors.

Crude incidence rates of acute liver injury of each drug or class showing a statistically significant association were computed using the number of patients exposed or prescriptions (as a surrogate marker of person-time) during the follow-up period as denominator and the number of cases among current users of that drug (or class) as numerator. The attributable risk percent was also calculated using the formula: ARP = ((OR − 1)/OR) × 100.

Results

The study cohort was made up of 1636792 persons who were followed-up during a total of 5404705 person-years. The automated search yielded 6134 subjects with a first-ever occurrence of a liver-related code. Of these, 1022 were retained as potential cases and their medical records requested. We received valid information from 778 patients (76%); of the remainder, 134 (13%) were transferred out, 28 (3%) died and for 82 (8%) the GP declared he/she could not help. The medical records were reviewed by two physicians resulting in 656 patients excluded due to the following reasons: (1) only minor serum enzyme elevations (257; 39.2%); (2) the patient presented with exclusion criteria or primary causes for liver injury (235; 35.8%); (3) the diagnosis was not confirmed or the finding was coincidental or detected in a routine examination (123; 18.8%); and (4) the patient was not referred to a hospital or a specialist for an additional work-up (40; 6.1%) (in one additional case there was a computer error). Six cases for which we did not receive the medical records were finally added after a second review of the patient profile, resulting in a total of 128 valid cases; these six cases presented all case definition criteria recorded on the computer files. The crude incidence rate of nonfatal, clinically relevant, idiopathic, acute liver disease was 2.4 (95%CI: 2.0, 2.8) per 100 000 person-years in our study population.

Thirty-six cases (28.2%) out of 128 were hospitalized as a consequence of the liver injury (crude incidence rate: 6.7 per one million person-years; 95%CI: 4.7, 9.2). One of them presented with fulminant hepatic failure resulting in a liver transplant. In 96 (75%) viral serological tests were performed with a negative result, in 77 (60%) an ultrasound scan was performed revealing no abnormality and, finally, in 17 (13%) patients a biopsy was conducted revealing liver injury with no specific aetiology. The functional pattern of liver injury was hepatocellular in 43 cases (34%), cholestatic in 40 (31%), mixed in 37 (29%) and it was not possible to determine in 8 (6%); 83 cases (65%) presented with jaundice (crude incidence rate: 15.4 per million person-years; 95%CI: 12.2, 19.0).

Cases and controls were perfectly matched for age, gender and calendar year. No relevant association was observed between acute liver injury and smoking (OR = 1.0; 95%CI: 0.7, 1.6) or alcohol intake (10 units or more *vs.* none: OR = 1.2; 0.7, 2.0). A moderate association with BMI ≥ 30 kg^{-2} was suggested (OR = 1.4; 0.8, 2.4). Of the 128 cases, 92 were exposed to a drug (excluding topical use) in the 15-day time window preceding the index date. All drugs with at least two cases exposed were analyzed. Those presenting an OR > 2 after adjustment for age, sex, calendar year, smoking, alcohol intake and BMI (first model) were further evaluated with additional adjustment for concomitant hepatotoxic medication (second model) (Table 1). According to the results obtained drugs were classified in three groups: (1) group I: drugs showing a statistically significant association in both models; (2) group II: drugs showing a statistically significant association only in the first model; and (3) group III drugs not reaching statistical significance in any model (Table 1). Group I included the antipsychotics chlorpromazine (AOR; 95% CI = 416; 45, 3480) and sulpiride (three cases, 0 controls; but two of the cases were exposed to chlorpromazine as well), the antibiotics amoxicillin/clavulanic acid (AOR = 94.8; 27.8, 323), flucloxacillin (AOR = 17.7; 4.4, 71.0), macrolides (AOR = 6.9; 2.3, 21.0), and tetracyclines (AOR = 6.2; 2.4, 15.8); the antiemetic metoclopramide (AOR = 6.2; 1.8, 21.3); the sedative antihistamine chlorpheniramine (AOR = 9.6; 1.9, 49.7); the drug for Ménière's disease betahistine (OR = 15.3; 2.9, 80.7); the drug for the inflammatory bowel disease sulphasalazine (OR = 25.5; 6.0, 109); the immunosupressant azathioprine (OR = 10.5; 1.4, 76.4), the antiepileptics (AOR: 5.1; 1.9, 13.7) and the NSAID diclofenac (OR = 4.1; 1.9, 8.8).

The results of our study are compatible with a lack of relevant hepatotoxic effect (AOR point estimate around 1 and upper 95% CI limit less than 3), with the following drugs/classes: low-dose aspirin, NSAIDs other than diclofenac, calcium channel blockers, β-adrenoceptor blockers, hormone replacement therapy, ACE inhibitors, thiazides and inhaled corticosteroids (Table 2) (the latter group was used as a negative internal control). For nitrates (4 cases, 90 controls; AOR = 1.5; 0.5, 4.3), levothyroxine (4 cases, 104 controls; AOR = 1.5; 0.5, 4.3), hypnotics (2 cases, 86 controls; AOR = 0.8; 0.2, 3.5), proton pump inhibitors (2 cases, 81 controls; AOR = 0.9; 0.2, 3.9) and oral contraceptives (4 cases; 107 controls; AOR = 1.6; 0.4, 5.5) an increased risk

Table 1
Risk of acute liver disorder and current use of drugs. Only shown those drugs with at least a moderate association (OR > 2) in the first model

Therapeutic class/Drugs	Cases ($n = 128$)	Controls ($n = 5000$)	OR* (95% CI) First model	AOR$ (95% CI) Second model
Group I #				
Chlorpromazine	6	1	302 (35, 2593)	416 (45, 3840)
Sulpiride	3	0	∞	–
Amoxicillin/clavulanic acid				
current use	8	5	86.7 (27, 278)	94.8 (27.8, 323)
recent use	5	8	27.1 (8.5, 85.9)	31.9 (9.8, 104)
Sulphasalazine	4	5	34.0 (8.8, 132)	25.5 (6.0, 109)
Flucloxacillin	4	9	20.0 (5.9, 67.4)	17.7 (4.4, 71.0)
Betahistine	2	8	11.1 (2.3, 54.6)	15.3 (2.9, 80.7)
Azathioprine	2	4	23.8 (4.1, 137)	10.5 (1.4, 76.4)
Chlorpheniramine	3	6	22.1 (5.2, 93.3)	9.6 (1.9, 49.7)
Macrolides	6	19	14.6 (5.6, 38.2)	6.9 (2.3, 21.0)
Erythromycin	4	16	11.5 (3.7, 35.8)	5.3 (1.4, 19.9)
Clarithromycin	2	3	17.1 (3.0, 97)	6.1 (0.8, 45.9)
Tetraciclines	6	41	5.9 (2.4, 14.5)	6.2 (2.4, 15.8)
Metoclopramide	5	13	14.3 (4.9, 41.5)	6.2 (1.8, 21.3)
Antiepileptics	5	42	5.0 (1.9, 12.9)	5.1 (1.9, 13.7)
Valproic acid	2	9	8.4 (1.7, 40.6)	9.7 (1.9, 50.7)
Carbamazepine	3	18	7.9 (2.3, 11.8)	5.4 (1.4, 20.4)
Diclofenac	10	87	5.7 (2.8, 11.8)	4.1 (1.9, 8.8)
Group II #				
Trimethoprim	2	18	4.8 (1.1, 21.2)	2.9 (0.6, 14.1)
Mebeverine	2	16	4.5 (1.0, 20.1)	2.2 (0.4, 12.6)
Tricyclic antidepressants	8	117	2.9 (1.4, 6.3)	1.8 (0.8, 4.2)
Amoxicillin	6	61	3.8 (1.6, 9.3)	1.7 (0.6, 4.8)
Anxiolytics	4	53	3.4 (1.2, 9.5)	1.7 (0.5, 5.3)
Cephalosporins	2	20	4.2 (1.0, 4.2)	1.5 (0.3, 8.1)
Paracetamol (alone or combined)	12	267	2.1 (1.1, 4.1)	1.0 (0.5, 2.0)
Group III #				
SSRIs	3	49	2.4 (0.7, 7.7)	2.2 (0.6, 7.5)
Allopurinol	3	48	2.3 (0.7, 7.6)	2.1 (0.6, 7.3)
Loop diuretics	5	86	2.2 (0.8, 5.7)	1.2 (0.4, 3.3)
Statins	4	62	2.3 (0.8, 6.5)	1.4 (0.5, 4.4)
Cimetidine	3	53	2.0 (0.6, 6.6)	1.3 (0.3, 5.0)
Oral antidiabetics	3	39	2.4 (0.7, 8.3)	1.3 (0.3, 5.1)
Codeine/dihydrocodeine (alone)	3	26	3.3 (0.8, 14.3)	1.1 (0.3, 4.3)
Systemic corticosteroids	3	48	2.5 (0.8, 8.3)	0.8 (0.2, 3.0)

**First model: Adjusted for age, sex, calendar year, smoking, alcohol intake and BMI; $Second model: OR adjusted in addition for the presence of other potentially hepatotoxic drugs (those showing an OR > 2 in first analysis). Drugs were grouped in four variables (NSAIDs/analgesics, antibiotics, psychotropic/neurologic drugs, and miscellaneous). The drug or class whose effect was explored was withdrawn from the corresponding grouped variable each time. #Group I: Drugs showing a relevant association in both models; Group II: Drugs showing a significant association in the first but not in the second model; Group III: Drugs not showing a significant association in any model. Note: 12 cases were exposed to more than one drug/class of the Group I in the 15-days time window before the index date.*

Table 2
Drugs for which a relevant association with an increased risk of acute liver injury (upper 95% CI limit less than 3) can be excluded

Therapeutic class/Drugs	Cases ($n = 128$)	Controls ($n = 5000$)	OR* (95%CI)	AOR# (95% CI)
Inhaled corticosteroids	4	147	1.0 (0.4, 2.7)	0.4 (0.2, 1.3)
Thiazides	5	194	0.9 (0.4, 2.3)	0.9 (0.4, 2.4)
ACE inhibitors	4	161	0.8 (0.3, 2.3)	0.7 (0.2, 2.0)
HRT	5	227	0.8 (0.3, 2.1)	0.7 (0.3, 1.8)
β-adrenoceptor blockers	5	290	0.6 (0.3, 1.6)	0.4 (0.2, 1.1)
NSAIDs$ (excluding diclofenac)	2	170	0.6 (0.1, 2.3)	0.4 (0.1, 1.5)
Calcium channel blockers	3	203	0.5 (0.2, 1.6)	0.3 (0.1, 1.0)
Low-dose aspirin	2	149	0.4 (0.1, 1.8)	0.2 (0, 0.8)

**Adjusted for age, sex, calendar year, smoking, alcohol intake, and BMI. # Adjusted in addition for hepatotoxic drugs. $Both cases were exposed to naproxen as compared with 29 controls; AOR = 1.7 (0.3, 9.1). Controls were also exposed to ibuprofen (73), indometacin (16), ketoprofen (12), piroxicam (8), mefenamic acid (8), fenfuben (5), meloxicam (4) and others (15) Note: One case and 60 controls had an antecedent of heart failure. The exclusion of such patients did not materially change the results.*

Table 3
Functional patterns and presence of jaundice in acute liver injury associated with drugs shown as hepatotoxic in the present study

Drug/Class	Total	Hepatocellular	Cholestatic	Mixed	Unknown	Jaundice (%)
Amoxicillin/clavulanic acid	13	4	3	5	1	10 (77)
Flucloxacillin	4	0	1	2	1	3 (75)
Tetraciclines	6	2	1	3	0	4 (67)
Macrolides	6	1	3	0	2	2 (33)
Chlorpromazine	6	0	5	1	0	6 (100)
Sulpiride	3	1	2	0	0	2 (67)
Antiepileptics	5	3	0	1	1	1 (20)
Tricyclic antidepressants	8	4	2	1	1	7 (88)
Sulfasalazine	4	1	3	0	0	1 (25)
Azathioprine	2	0	0	2	0	0 (0)
Metoclopramide	5	2	3	0	0	4 (80)
Chorpheniramine	3	1	2	0	0	2 (67)
Betahistine	2	0	1	1	0	2 (100)
Diclofenac	10	2	4	3	1	3 (30)

cannot be excluded but the results suggest only a moderate effect, if any.

The functional pattern and presence of jaundice in acute liver injury associated with drugs showing a statistically significant association after full adjustment are described in Table 3. Within the group of drugs showing a significant association with acute liver injury we assessed the effect of being exposed to more than one hepatotoxic drug. As shown in Table 4, the risk substantially increased by a factor of 6.

A dose effect was only apparent for diclofenac (<150 mg: AOR = 2.7; 0.8, 9.5; ≥150 mg: AOR = 5.1; 1.8, 13.9), amoxicillin/clavulanic acid (≤750 mg: AOR = 39.1; 15.8, 96.8; >750 mg: AOR = ∞; 3 cases and 0 controls) and flucloxacillin (≤1000 mg: AOR = 11.6; 2.0, 68.4; >1000 mg: 42.2; 4.1, 432).

For most hepatotoxic drugs the association was only or mainly observed during the course of the first prescription (7 days for antibiotics, 30 days for other drugs) (see Table 5). Of note, a short-term effect was also

Table 4
Effect of combining two or more potentially hepatotoxic drugs on the risk of acute liver injury

	Cases (n = 128)	Controls (n = 5000)	OR*(95% CI)
Non use	15	1363	1 (ref.)
Current use$	58	266	20.1 (11.1, 36.4)
1 drug	46	252	16.7 (9.1, 30.6)
2 + drugs	12	14	98.7 (37.9, 257)
Past use	55	3371	1.5 (0.8, 2.6)

**Adjusted for age, sex, calendar year, smoking, alcohol intake and BMI; $The following drugs were included: Diclofenac, amoxicillin/clavulanic acid (including current and recent users), flucloxacilline, tetracyclines, macrolides, chlorpromazine, sulpiride, antiepileptics, metoclopramide, chlorpheniramine, betahistine, sulfasalazine and azathioprine. The exclusion of metoclopramide and chlorpheniramine hardly changed the results: 1 drug, OR = 17.7, 9.7, 32.1; 2 + drugs: OR = 92.8, 31.5, 27.8.*

Table 5
Drugs showing a short-term effect on risk of acute liver injury

	Cases (*n* = 128)	Controls (*n* = 5000)	AOR* (95% CI)
Chlorpromazine			
≤30 days	5	0	∞
>30 days	1	1	–
Antiepileptics			
≤30 days	2	3	33.2 (4.4, 248)
>30 days	3	39	3.2 (0.9, 11.1)
Tricyclic antidepressants			
≤30 days	4	12	12.2 (3.4, 43.5)
>30 days	4	105	0.9 (0.3, 2.7)
Amoxicillin/clavulanic acid†			
≤7 days	11	9	67.8 (26, 176)
>7 days	2	4	23.8 (3.8, 148)
Tetracyclines			
≤7 days	2	6	20.2 (3.8, 108)
7 days	4	35	4.1 (1.3, 13.1)
Macrolides			
≤7 days	6	13	9.9 (3.0, 32.5)
>7 days	0	6	–
Metoclopramide			
≤30 days	4	6	11.6 (2.8, 47.5)
>30 days	1	7	1.1 (0.1, 12.1)
Chlorpheniramine			
≤30 days	3	4	20.3 (3.4, 122)
>30 days	0	2	–

**Adjusted for age, sex, calendar year, smoking, alcohol intake, BMI and other hepatotoxic drugs; †Including current and recent users*

observed with tricyclic antidepressants, a group that fell within the drugs not showing an association after full adjustment for the presence of other hepatotoxic drugs. A greater effect associated with longer duration of treatment was observed for sulphasalazine (≤30 days: no cases; >30 days: AOR = 36.6; 7.8, 171), flucloxacillin (≤7 days: AOR = 4.5; 0.5, 44; >7 days: AOR = 92; 12, 696), and diclofenac (≤90 days: AOR = 1.9; 0.4, 8.6; >90 days: AOR = 5.5; 2.3, 13.1). Amoxicillin/clavulanic acid (current and recent users combined) presented an excess risk both at short (≤7 days: AOR = 67.8; 26, 176) and long-term treatment (>7 days: AOR = 23.8; 3.8–148); in five cases exposed to amoxicillin/clavulanic acid, the reaction began to appear 20 days or more after the recorded end of the prescription (recent users).

The role of age (≤60, >60 years) and sex as effect modification factors was explored on the therapeutic groups (NSAIDs/analgesics, psychotropic/neurologic drugs, antibiotics and miscellanea) and on individual drugs. Broadly, no remarkable effect was observed for most drugs (data not shown). A higher risk was suggested in women for macrolides (females: AOR = 15.8; 3.2, 78.2; males: AOR = 3.9; 0.7, 21.6) and diclofenac (females: AOR = 9.2; 2.9, 28.6; males: AOR = 2.5; 0.8, 7.9). Regarding amoxicillin/clavulanic acid the odds ratio for both current and recent users combined increased to the same extent for both older (AOR = 54.4; 8.4, 352) and younger people (AOR = 60.9; 22.9, 262) and for both males (AOR = 66.0; 22.3, 195) and females (AOR = 58.1; 13.5, 250).

Crude incidence rates for those drugs showing a definite association with acute liver injuries in the case-control adjusted analysis appear in Table 6, along with their corresponding attributable risk percent.

Discussion

The results of our study show that idiopathic, acute and clinically relevant liver injury, which has the use of drugs as the most probable aetiology, is a very rare event in the general population, confirming recent estimations from other studies [8, 9]. For most cases, a previous exposure to at least one drug for systemic use was identified, though the association was only epidemiologically confirmed for a few drugs. The combination of two or more hepatotoxic drugs considerably increases the

Table 6
Crude incidence rates of acute liver injury for those drugs showing a significant association in the case-control analysis

Drug/Class	Cases	Users	Prescriptions	P/U§	Incidence x 100 000 users (95% CI)	Incidence rate x 100 000 prescriptions* (95% CI)	Attributable risk (%)
Antibiotics							
Amox/clav†	13	151 942	225 249	1.5	8.6 (2.4, 14.6)	5.8 (1.6, 9.9)	99
Flucloxacillin	4	155 185	224 009	1.4	2.6 (0.7, 6.6)	1.8 (0.9, 4.6)	94
Tetraciclines	6	162 417	401 555	2.5	3.7 (1.4, 8.0)	1.5 (0.5, 3.3)	84
Macrolides	6	243 832	447 064	1.8	2.5 (0.9, 5.4)	1.3 (0.5, 2.9)	86
Central Nervous System							
Sulpiride$	3	1 241	16 818	13.6	241.7 (49.9, 706.3)	17.8 (3.7, 52.1)	100
Chlorpromazine	6	4 432	56 829	12.8	135.4 (49.7, 294.6)	10.6 (3.9, 22.9)	100
Carbamazepine	3	13 676	203 220	14.9	21.9 (4.5, 64.1)	1.5 (0.3, 4.3)	77
Sodium valproate	2	6 435	132 881	20.6	31.1 (3.8, 112.0)	1.3 (0.2, 5.4)	92
TCAs#	8	98 349	813 745	8.3	8.1 (3.5, 16.0)	1.0 (0.4, 1.9)	44
Miscellaneous							
Sulfasalazine	4	5 335	70 761	13.3	75.0 (20.4, 191.9)	5.7 (1.5, 14.5)	96
Azathioprine	2	2 204	35 685	16.2	90.7 (11.0, 327.7)	5.6 (0.6, 20.2)	90
Metoclopramide	5	41 689	90 535	2.2	12.0 (3.9, 28.0)	5.5 (1.8, 12.9)	84
Chlorpheniramine	3	43 137	79 396	1.8	7.0 (1.4, 20.3)	3.8 (0.8, 11.0)	90
Betahistine	2	15 780	74 929	4.7	12.7 (1.5, 45.8)	2.7 (0.3, 9.6)	93
Diclofenac	10	157 721	582 993	3.7	6.3 (3.0, 11.7)	1.7 (0.8, 3.2)	76

*§Prescriptions per user; *For comparison it should be taken into account that a prescription of an antibiotic is on average of 7-days duration, while for other drugs the duration is normally about 30 days or more. †Combination amoxicillin/clavulanic acid. Including current and recent users; $Two of the three cases were also exposed to chlorpromazine; #Tricyclic antidepressants.*

risk of suffering an acute liver injury. Clinicians should bear this in mind at the time of prescribing.

Quantitative information about the absolute and relative risks of acute liver injury associated with drugs is scanty, even for the older drugs. Over the nineties several authors using automated healthcare databases have provided figures of incidence of drug-related acute liver injury [10], but the information refers to a limited number of drugs, the relative risks were usually obtained by comparison with cohorts of people exposed to other drugs, and most of them included study periods before 1995. In the present study we have been able to follow-up a large population over a much more proximate study period and estimate the association with up to 40 different drugs or classes using a case-control approach nested in a well-defined study cohort.

The epidemiologic studies performed up to now have consistently estimated an absolute risk of about 1 per 100 000 prescriptions (9 per 100 000 person-years) for NSAIDs as a group [11]. Our results confirm this very low risk of acute liver injury associated with NSAIDs (0.7 per 100 000 prescriptions) but cast doubts on the widespread assumption of a class-effect, as only diclofenac is identified with an excess risk. When diclofenac is excluded, only two cases exposed to other NSAIDs (both to naproxen) can be identified leading to an OR of 0.6 (upper 95% CI: 2.3) and a crude incidence rate of 0.2 per 100 000 prescriptions which most probably is close to the baseline risk. The fact that all the two cases were exposed to naproxen, however, leaves room for uncertainty about the hepatotoxic potential of this drug. Other studies have singled out sulindac as having a particularly high-risk of acute liver injury [11–13]. We were not able to confirm this finding because there was no single case nor control exposed to sulindac, indicating a very low consumption in our study population. Diclofenac has since long been recognized as having a particular hepatotoxic potential among the NSAIDs [3, 14, 15], although no epidemiologic study until now has been able to confirm this point [10–12, 16]. Noteworthy, the increased risk we found associated with diclofenac appeared particularly associated with higher doses (150 mg or over) and long-term treatments (over 90 days). Moreover, most cases presented without jaundice. No specific functional pattern seemed to prevail.

Our research confirms what has been described elsewhere about the epidemiology of chlorpromazine-induced acute liver injury [2, 13, 15, 17, 18]: (1) its predominant cholestatic pattern, (2) its dose-independence (the dose ranged from 25 to 150 mg in our cases), (3) the usual clinical presentation with jaundice, (4) the short time to onset (most cases occurred within the first month of therapy) and, finally (5) an incidence of about 1 per 1000 users, the highest we have found for an individual drug. The apparent high risk associated with sulpiride in our study cannot be properly evaluated as two of the three cases detected were also exposed to chlorpromazine, but raises doubts about its hepatotoxicity, that have been reported in isolated cases [19].

Tricyclic antidepressants are among the drugs considered as potentially hepatotoxic, but only anecdotal cases have been reported in the literature [2, 15, 17]. We found a statistically significant association but only for short-term exposure (less than 30 days). All functional patterns were present, but most cases presented with clinical jaundice. The attributable risk was roughly 4 in 100 000 users. Recently, there has been much debate about the association of liver injury with the new selective serotonin reuptake inhibitors [20]. Until now, no epidemiological study has shown an association. Our results suggest a moderate increased risk (though statistical significance was not reached).

Carbamazepine is structurally related with tricyclic antidepressants, but its hepatotoxic potential has been considered much stronger [2, 13, 15, 21]. Our results confirm this view. The main functional pattern appears to be hepatocellular with an estimated incidence of 1 per 5000 users. A similar picture was found for sodium valproate, though the small number of exposed cases impedes a closer evaluation.

Among antibiotics, the combination of amoxicillin and clavulanic presents the strongest association of all. As previously described [22], the functional pattern is predominantly cholestatic or mixed with most patients presenting with jaundice. The risk is high both at short- and long-term duration of use and a dose-dependence is clearly suggested. A late onset after the end of treatment was observed for almost half of cases. Contrary to a previous study [23], we did not find an age effect, nor did sex behave as an effect modification factor. The incidence was close to 1 per 10 000 users, slightly lower than the one previously estimated [23]. Although amoxicillin was found to be associated with a small increased risk in the first analysis, such association vanished after full adjustment for the presence of other potential hepatotoxic drugs. This result supports the well-accepted idea of amoxicillin as essentially a nonhepatotoxic drug [2, 15, 23, 24] emphasizing the role of the clavulanic acid moiety of amoxicillin/clavulanic acid as the main culprit for the liver injury.

The liver toxicity of flucloxacillin is well-established. Firstly suggested by individual case reports and then confirmed in epidemiological studies [2, 15, 25]. Our results support the existence of a strong association. The predominant functional pattern appears to be cholestatic, with most patients presenting with jaundice. Dose and duration effects are clearly suggested and the estimated incidence is about 1 per 39 000 users (1 per 50 000 prescriptions), lower than the one previously described [2, 25]. The warning about risk factors (age, long-duration) may have had an impact reducing the risk.

Our results suggest that the new macrolide clarithromycin may share the hepatotoxic profile of erythromycin, confirming some case-reports [26]. The reaction associated with macrolides appeared to be rather short-term, predominantly cholestatic and with an incidence of 1–2 per 50 000 users. No specific salt of erythromycin was involved. Likewise, tetracyclines (oxytetracycline and minocycline) were associated with an increased risk of hepatic injury, with a predominant cholestatic pattern, clinical presentation with jaundice, and rather low incidence (1 per 25 000 users).

Sulphasalazine and azathioprin are among the most hepatotoxic drugs we found. Both are well-recognized in the literature a such [2, 13, 15, 27–29]. The predominant pattern is cholestatic or mixed and the incidence is close to 1 per 1000 users. All sulphasalazine associated cases occurred after the first month of treatment.

The associations we found with metoclopramide, chlorpheniramine and betahistine were rather unexpected. Although there are isolated cases of liver injury reported in the literature [29, 30], they are not considered hepatotoxic drugs [2, 15]. The two cases associated with betahistine occurred after long exposure, while most associated with metoclopramide and chlorpheniramine appeared shortly after the beginning of treatment (7 days or less). Moreover, the cases related with the two latter drugs had other well-recognized hepatotoxic drugs used concomitantly: flucloxacillin (two cases), chlorpromazine, sulpiride and diclofenac/gold compounds. Those facts and the standard indications of both drugs (vomiting and pruritus, respectively), that may be early symptoms of an evolving acute liver injury, lead to think to confounding by indication as an alternative explanation of the strong association found for both drugs.

The lack of association with paracetamol alone or combined is noteworthy, indicating that at doses nor-

mally used paracetamol is a nonhepatotoxic agent in the general population (lacking other risk factors as high alcohol intake).

The strengths of the present study are the following: (1) the case-control analysis has been nested in a well-defined cohort allowing a true random sample for controls which assures the representativeness of the exposure; (2) the large population followed up, which makes this study the largest ever performed in this area; (3) the recording of all prescriptions in the computer, which eludes the recall problem of the traditional interview-based case-control studies; (4) the strict criteria applied for the case ascertainment and validation; (5) the blindness of the researchers who ascertained the cases with respect to the exposure status; and the (6) the full adjustment for all potential hepatotoxic drugs.

The weaknesses of the study are the following: (1) over the counter (OTC) drugs, or hepatotoxic substances not considered as medicinal products like herbal medicines, are not recorded in a systematic way on the computer; (2) the study was retrospective and some of the cases were lacking a complete diagnostic work-up; (3) clinical records were not available for patients who died, therefore our results only refer to nonfatal liver injury; (4) the use of idiopathic cases could be criticised, as a drug may also be the cause of liver injury in the presence of other predisposing factors (e.g. heavy alcohol consumption); we agree with this but the inclusion of such cases could also have been put into question by others alleging they are exposed to potential confounding factors; and (5) despite the large population followed, the final number of cases was rather limited for certain analysis. It is improbable, however, that these limitations may have had a relevant impact on the validity of the study.

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Francisco J. de Abajo conceived the study, took part in the case review process, performed the main analysis of the data and wrote the first version of the paper, Dolores Montero coordinated the case review process, helped in the analysis and contributed significantly to the writing of the final version, Mariano Madurga helped in the validation process, performed the literature search of hepatotoxic drugs, helped in the analysis and contribute to the interpretation of the data and writing of the paper, Luis A. García Rodríguez contributed significantly to the study design, case review, analysis and writing and performed all the work with the raw data provided by the General Practice Research Database.

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